411. A method according to Claim 410 wherein the amount of said ultrasound energy is also sufficient to stimulate lysis of said thrombus.--

REMARKS

Reconsideration of the present application in view of the above amendments and following remarks is requested respectfully.

Status of the Claims

After entry of the amendments, Claims 100, 102, 103, 113, 115, 122, 124, 127, 194 to 200, 203, 210 to 238, 245 to 248, 255 to 270, 277 to 280, 287 to 292, 294 to 300, 303, 310 to 329, 331 to 337, and 347 to 411 are pending in the present application. Claims 101, 114, 123, 201, 202, 204 to 209, 239 to 244, 249 to 254, 271 to 276, 281 to 286, 293, 301, 302, 304 to 309, 330, and 338 to 346 have been canceled. Claims 100, 102, 113, 115, 122, 124, 127, 194, 203, 229, 236, 261, 262, 268, 294, 303, 331 and 351 have been amended. Claims 357 to 411 are newly added.

The Amendments

The claims have been amended to clarify that the compositions, processes, methods and formulations of the claimed invention are directed to lipid vesicles. Independent Claims 100 and 127 have been amended to clarify that the lipid vesicles bear a targeting ligand, which is covalently bound to the lipid vesicle via a hydrophilic polymer linking group. The use of a hydrophilic polymer linking group to covalently bind a targeting ligand to the vesicle is described, for example, at page 26, lines 1 to 10 and page 85, lines 1 to 5 of the present application. Independent Claims 113 and 122 have been amended to recite a targeted formulation, and process for making that formulation, wherein the targeting ligand comprises the sequence Lys-Gln-Ala-Gly-Asp-Val (SEQ ID NO 1). The use of this targeting ligand is supported in the disclosure, for example, at page 67, lines 6 to 9. Newly added claims 357 to 411 recite a targeted formulation, and methods of using that formulation for *in vivo* delivery of a bioactive agent, that also make use of that same targeting ligand.

Priority

In the Office Action dated July 18, 2000, the Examiner indicated that the effective priority date used for examination is December 22, 1998, which represents the filing date of the instant application. Basically, the Examiner has asserted that the priority applications fail to teach compositions comprising gaseous lipid vessels in combination with a targeting moiety such as Arginine-Glycine-Aspartic acid directed to a glycoprotein receptor such as GPIIbIII, or methods of lysing a thrombus, comprising administering to a patient a targeted vesicle

composition encapsulating a gas, and scanning the patient with ultrasound. Applicants respectfully traverse this finding.

As indicated on page one of the instant disclosure, this application is a continuation-in-part of U.S. application Serial No. 08/660,032, filed June 6, 1996, which is a continuation-in-part of U.S. application Serial No. 08/640,464, filed May 1, 1996, which in turn is a continuation-in-part of U.S. application Serial No. 08/497,684, filed June 7, 1995. The disclosures of each of those foregoing applications was incorporated by reference, in their entirety, into the present application. Thus, any claims of the present application which are supported by one of those priority applications may rely on the filing date of such application as the effective filing date for examination.

The Examiner's attention is respectfully drawn to U.S. Application Serial No. 08/640,464 ("the '464 application"), filed May 1, 1996. This application is generally directed, as the title suggests, to targeted compositions for diagnostic and therapeutic use. At page 8, for example, embodiments of the invention are described which comprise lipid vesicles which encapsulate a fluorinated gas, in combination with a targeting ligand, wherein the targeting ligand targets cells or receptors selected from the group consisting of myocardial cells, endothelial cells, epithelial cells, tumor cells and the glycoprotein GPIIbIIIa receptor. The use of perfluorocarbon gases and gaseous precursors is described, for example, at page 31, lines 17 to 27. The use of the tripeptide Arginine-Glycine-Aspartic acid (RGD) as a targeting ligand is described, for example, at page 49, lines 1-2, and page 52, lines 18 to 26. Use of the targeting ligand Lysine-Glutamine-Alanine-Glysine-Aspartic acid-Valine is described in Example 4, on

page 102. The use of hydrophilic polymers to link the targeting ligand to the lipid vesicles is also described in that example and elsewhere in the application, for example, at page 58, lines 16 to 33. The application of ultrasound following treatment with the targeted formulations to promote thrombolysis is described, for example, at page 68, lines 13 to 18. Thus all of the elements referred to by the Examiner can be found in this priority document. Accordingly, Applicants respectfully request that the filing date of the '464 application, May 1, 1996, be utilized as the effective filing date for examination of the present claims.

Rejection under Section 102(e)

Claims 100 to 103, 113 to 115, 122 to 124, 194 to 201, 210, 214 to 215, 217 to 20, 223 to 233, 236, 245, 249 to 252, 255 to 268, 277, 281 to 284, 287 to 301, 310, 314 to 317 and 320 to 338 stand rejected under 35 U.S.C. 102(e) as being anticipated by Lanza et al., U.S. Patent No. 5,989,520 ("Lanza").

As a preliminary matter, Applicants note that once the instant claims are properly accorded an effective filing date of May 1, 1996, Lanza is no longer a proper 102(e) reference to the instant application. In this regard, Applicants note that both Lanza (filed February 19, 1998) and its parent, U.S. Application Serial No. 08/647,277 (filed May 23, 1996), were filed after the effective filing date of the instant application. Applicants recognize, however, that Lanza, on its face, claims priority back to U.S. Application Serial No. 08/488,743, now U.S. Patent No. 5,690,907 ("the Lanza '907 patent"), which does have a filing date prior to the effective filing

date of the instant claims. Accordingly, Applicants will address their comments to the Lanza '907 patent, rather than the later Lanza reference cited by the Examiner.

Summary of the Invention

The present invention is generally directed to targeted formulations comprising lipid vesicles having a fluorinated gas encapsulated therein, and methods of making and using those formulations. In certain embodiments, the vesicles bear a targeting ligand, which is covalently bound to the vesicle via a hydrophilic polymer linking group. In certain other embodiments, the formulations comprise, in combination with the lipid vesicles encapsulating the fluorinated gas, a bioactive agent and a targeting ligand which comprises the sequence Lys-Gln-Ala-Gly-Asp-Val (SEQ ID NO 1).

Summary of the Lanza '907 Patent

The Lanza '907 patent is generally directed to a method for ligand-based binding of lipid encapsulated particles to molecular epitopes on a surface comprising sequentially administering (a) a site-specific ligand activated with a biotin activating agent; (b) an avidin activating agent; and (c) lipid encapsulated particles activated with a biotin activating agent, whereby the ligand is conjugated to the particles through an avidin-biotin interaction so that the resulting conjugate is bound to the molecular epitopes. *See* Lanza '907 abstract. Perfluorcarbon emulsions may be encapsulated with the lipid particles, and the emulsions may generate gaseous vapors. *See* col. 6, lines 59 to 62. Lanza further describes both diagnostic and therapeutic applications for this system. *See* col. 7, lines 7 to 67.

Lanza '907 Does Not Anticipate Applicant's Claimed Invention

Applicants claims distinguish over the Lanza '907 patent by defining embodiments that either utilize a targeting ligand that is bound to the lipid vesicle *via a hydrophilic polymer linking group* (e.g. independent Claims 100, 127) or which utilize a *targeting ligand comprising the sequence Lys-Gln-Ala-Gly-Asp-Val* (e.g. independent Claims 113, 122, 357 and 383). There is simply no teaching in the Lanza '907 patent of the of either the linking groups or targeting ligands recited in Applicants' claims.

Nor is Applicant's invention a merely obvious variation of the system described by Lanza. Lanza's tri-phasic, sequential administration procedure (*see* col. 4, lines 44 to 56) is clearly completely different than the methods of the present invention, which mention no such tri-phasic administration. Additionally, although Lanza does indicate that the dense perfluorochemical emulsions[1] utilized in his lipid particles may evolve a gas (*see* col. 6, lines 57 to 62), the application does not teach the use of any fluorocarbon gases *per se*, that is, fluorocarbon compounds which would be gases at room or body temperature. Clearly, Lanza is utterly and completely silent regarding the specific gaseous fluorocarbons recited, for example, in dependent Claims 211 to 216. Accordingly, Applicants respectfully submit that there is

[1]Applicants note that many of the compounds referred to as "perfluorocarbons" by Lanza are not true perfluorocarbons, i.e. hydrocarbon compounds in which all of the hydrogen atoms have been replaced with fluorine atoms, and all are dense liquids at room or body temperature. For example, Lanza refers to perfluorotributylamine, perfluorooctylbromide, perfluorodichlorooctane, perfluorotripropylamine and perfluorotrimethylcyclohexane as "perfluorocarbons." *See* col. 6, lines 2 to 9. Applicants' definition of the term "perfluorocarbon" as referring to fully fluorinated fluorocarbons (*see* page 44, line 6), does not encompass such compounds.

nothing in Lanza which would lead one of ordinary skill in the art to the methods, compositions, formulations and processes defined by Applicants' claims. Applicants, therefore, respectfully request that the rejection under Section 102 be withdrawn.

Rejection under Section 103(a)

Claims 100 to 103, 113 to 115, 122 to 124, 127, 194 to 203, 210 to 238, 245 to 270, 277 to 303, 310 to 340 and 347 to 356 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Nishioka et al, *J. Am Cardiol.* 1997; 30:561-8 ("Nishioka"), in view of Lanza, Schutt, et al., U.S. Patent No. 5,626,833 ("Schutt"), Konigsberg et al., U.S. Patent No. 5,258,499 ("Konigsberg"), EP 0 422 938 ("Nutt"), and Ishihara, U.S. Patent No. 5,1990,766.

Applicants respectfully submit that once the proper effective filing date of the instant claims is recognized, this rejection will be rendered moot. As discussed in depth above, the claims of the instant application are entitled to an effective filing date of May 1, 1996. Thus, Applicants respectfully submit that Nishioka, which was published in August, 1977, like Lanza, as discussed above, is not prior art to the instant application. Since the Examiner has not indicated that the secondary references, either alone, or standing alone or in combination with each other, but not in combination with Nishioka, render the invention unpatentable, removal of Nishioka from consideration obviates the rejection. Accordingly, Applicants respectfully request that the rejection under Section 103 be withdrawn.

Even if the May 1, 1996 priority date for the instant claims is not recognized, however, Applicants respectfully submit that the combination of references cited by the

Examiner does not teach or suggest the invention defined by the amended claims. Specifically, none of the combined references teach the use of targeted formulations comprising lipid vesicles which encapsulate a fluorinated gas, in combination with a bioactive agent, *wherein the lipid vesicles bear a targeting ligand covalently bound to the vesicle by a hydrophilic polymer linking group* or where the *targeting ligand comprises the sequence Lys-Gln-Ala-Gly-Asp-Val*, as recited in the amended claims.

Nishioka teaches that administration of a contrast agent comprised of a perfluoropentane (i.e. dodecafluoropentane) emulsion, followed by the application of transcutaneous ultrasound, may enhance ultrasound clot disruption without the use of a thrombolytic agent. *See* page 568, col. 1, ¶ 2. Nishioka does not describe the use of targeted formulations, although it does indicate that the use of a tissue (thrombus)-targeted contrast agent may be worth trying[2]. *See* page 567, col. 2, ¶ 3. Nishioka also does not describe the use of lipid vesicles to encapsulate the perflouropentane, or the use of such vesicles in combination with a bioactive agent, nor does Nishioka describe methods for the therapeutic delivery *in vivo* of a bioactive agent.

The teachings of Lanza in U.S. Patent No. 5,989,520, like those of the Lanza '907 patent discussed above, relate to administration of lipid particles which encapsulate a perfluorochemical emulsion. The lipid particles may be bound to a target surface through an

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[2]Nishioka only proposes this, and does not report ever having done so. Nishioka makes reference to the targeted contrast agents reported in Lanza, et al., "Specific Acoustic Enhancement of Vascular Thrombi *in vivo* With A Novel Site Targeted Ultrasonic Contrast Agent" *Circulation* 1995; 92 Supplement 1, 1-260,

avidin-biotin complex. *See* col. 4, lines 23 to 40. As discussed above in reference to the Lanza '907 patent, there is no mention in Lanza of the targeting ligand or linking groups recited in Applicants' claims. Nutt teaches alpha-amino acid containing chains of RGD that may inhibit binding of fibrinogen to the platelet membrane glycoprotein complex IIbIIIa receptor (*see* Nutt, page 11, lines 3 to 4), but contains no mention of a targeting ligand with the sequence Lys-Gln-Ala-Gly-Asp-Val. Konigsberg teaches the binding of targeting ligands to liposomes (*see e.g.*, abstract), but does not teach the use of liposomes which encapsulate a gas, the use of hydrophilic polymer linking groups to covalently bind the targeting ligand to the lipid vesicle, or the use of a targeting ligand with the sequence Lys-Gln-Ala-Gly-Asp-Val. Ishihara teaches the administration of drug carriers which are hollow microcapsules made of albumin, followed by application of ultrasound, in drug delivery methods (*see e.g.* Ishihara, Claim 5), but does not teach the use of lipid vesicles containing a fluorinated gas, or the use of targeting ligands.

Any combination of these references does not, therefore, teach or suggest the methods and compositions defined by Applicant's claims. None of the references teach the use of hydrophilic polymer linking groups for covalently binding the targeting ligand to the lipid vesicle, and none of the references teach the use of a targeting ligand with the sequence Lys-Gln-Ala-Gly-Asp-Val. Further combination of these references with Schutt does nothing to cure these deficiencies. Schutt, which is directed solely to diagnostic imaging, and not to therapeutic methods, contains no teaching or suggestion whatsoever of targeted formulations, much less of the linking groups or targeting ligands specified in Applicants' claims. Additionally, Applicants respectfully point out that the Examiner's statement that Schutt teaches

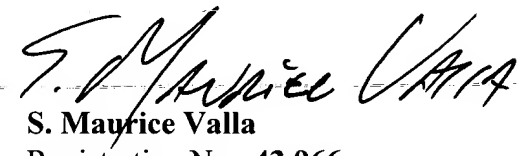
that use of the compositions disclosed therein can enhance the thrombolytic activity of agents such as TPA or Streptokinase (*see* page 8 of the Office Action) is incorrect. A more careful reading of Schutt indicates that the reference teaches that "*visualization of changes in myocardial tissue* due to or during various interventions, such as . . . use of thrombolytic agents (TPA or streptokinase) can also be enhanced." *See* Schutt, col. 11, lines 26 to 29 (emphasis added). Thus, Schutt does not teach the combined use of a microbubble contrast agent, a bioactive agent, and ultrasound to enhance thrombolysis, but only that the microbubble contrast agent disclosed therein may be useful for visualization of the changes induced by therapy with a thrombolytic agent.

From this discussion, it is clear that even if the proper effective filing date of the instant application is not recognized, the combined references cited by the Examiner do not teach or suggest the invention defined by Applicants' amended claims. Accordingly, Applicants again respectfully request that the rejection be reconsidered and withdrawn.

CONCLUSION

Applicants believe that the foregoing constitutes a full and complete response to the Office Action of record. Accordingly, an early and favorable Action is requested respectfully.

Respectfully submitted,



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